# Biophysics Rules the Cell Culture but Has Yet to Reach the Clinic: Why Is That?

Salomé Guillaumin, MSc
Ignacio Sallent, MSc
Dimitrios I. Zeugolis, PhD

Musculoskeletal injuries are the leading cause of physical disability worldwide, with associated annual direct and indirect healthcare expenditure in excess of $874 billion in the United States alone.[1] Current treatments are predominantly based on tissue grafts (autografts are preferred)[2,3] and biomaterials.[4,5] Given that the former are associated with scarce availability, insufficient remodeling, and adverse immune reactions,[6-8] and the latter with substandard stability, poor biologic response, and foreign body response,[9-11] their clinical suitability has been questioned and gave rise to the field of cell-based therapies.[12]

Cell-based therapies advocate that optimal repair and regeneration can be achieved through the utilization of the intrinsic capacity of cells to build native supramolecular assemblies; cells are the natural born extracellular matrix (ECM) builders, after all. Unfortunately, cell-based therapies require in vitro cell expansion in artificial tissue culture media and plastics. Removed from their optimal tissue niche, cells lose their phenotype, function, and therapeutic potency.[13,14] Thus, contemporary tissue engineering incorporates high levels of biomimicry in the design of functional and physiologically relevant in vitro microenvironments to recapitulate ex vivo, insofar as possible, the complexity of the in vivo tissue context of the cells. Here, we briefly discuss recent advancements in biophysical aspects of cell culture systems and whether these developments have influenced clinical translation and commercialization of cell-based therapies in the musculoskeletal space.

Biophysics and dynamics (in the form of architectural, geometrical, dimensional, and topographical features; biomechanical properties, such as elastic modulus and shear forces and cyclic strains; and localized density) are ubiquitous in nature and determine cell and tissue specificity and function.[15,16] For example, tendons are composed of highly ordered, bidirectionally aligned collagen fibrils (up to 100 nm to 1,000 nm in diameter), which, bundled together, form collagen fibers (1 μm to 20 μm in diameter) and collagen fiber bundles (20 μm to 500 μm in diameter).[17] Bone exhibits a radial gradient porous structure from the outside: the cortical bone has outer porosity of approximately 5%, while the inner part can reach porosity up to approximately 10%; porosity of the cancellous bone starts at approximately 50% in the outer layer and can reach approximately 90% in the inner layer.[18] Articular cartilage has a zonal architecture, and the organization and alignment of the collagen fibrils/fibers is different in every zone (eg, parallel, perpendicular, diagonal, radial).[19]

Advancements in engineering have made available numerous nano- and microfabrication technologies (eg, electrospinning, imprinting) that have enabled control of permanently differentiated cells and stem cells.[20,21] For example, electrospun and/or imprinted substrates have been shown not only to maintain tenocyte,[22,23] chondrocyte,[24] and osteoblast[25] phenotype, but also to direct stem cells toward tenogenic,[26] chondrogenic,[27] and osteogenic[28] lineages. The term durotaxis is used to describe the ability of cells to

From the Regenerative, Modular & Developmental Engineering Laboratory (REMODEL) and the Science Foundation Ireland (SFI) Centre for Research in Medical Devices (CÚRAM), Biomedical Sciences Building, National University of Ireland Galway (NUI Galway), Galway, Ireland (Mr. Guillaumin, Mr. Sallent, and Dr. Zaugolis).

*J Am Acad Orthop Surg* 2017;25: e144-e147

DOI: 10.5435/JAAOS-D-17-00324

migrate directionally toward areas of high ECM rigidity. ECM elasticity/mechanical compliance governs numerous in vivo biologic processes, including cellular spreading, migration, and differentiation; morphogenesis; wound healing; and disease progression.[29,30] In the last decade, numerous in vitro studies have demonstrated the positive influence of substrate rigidity in tendon-,[31] cartilage-,[32] and bone-derived[33] cell phenotype maintenance and in stem cell differentiation toward tenogenic,[34] chondrogenic,[35] and osteogenic[36] lineages. Static or dynamic uniaxial or multiaxial tensile, compressive, or shear mechanical loads are also crucial for the development, function, and healing of musculoekeltal tissues.[37,38] It is not a coincidence, after all, that exercise is an integral element of any orthopaedic rehabilitation regime.[39,40] Several bioreactor systems of variable complexity have been used as means to control tenocyte,[41] chondrocyte,[42] and osteoblast[43] phenotype in vitro and to direct stem cells toward tenogenic,[44] chondrogenic,[45] and osteogenic[46,47] lineages. Musculoskeletal tissues, like any other tissue, are highly dense ECM assemblies. Yet again, traditional cultures are conducted in dilute culture media that barely imitate the density of body fluids, let alone compact tissues.

To emulate this dense ECM microenvironment in vitro, macromolecular crowding, also known as localized density or excluding volume effect, has been proposed and has been shown to substantially modulate nuclear processes, such as gene transcription, RNA splicing and DNA replication, and protein properties, such as diffusion coefficients, folding kinetics, and thermodynamic activities, both intracellularly and extracellularly.[48,49] In vitro data have shown macromolecular crowding to maintain tenocyte and osteoblast phenotype[50] and to enhance chondrogenesis in stem cell culture.[51]

Despite the significant volume of work in the in vitro setting, only a handful of studies have assessed in preclinical models the influence of mechanical preconditioning in tissue regeneration. However, in all cases, the cells were seeded into/onto a scaffold, the cell/scaffold system was subjected to mechanical loading in vitro for a period of time, and then the cell/scaffold system was implanted.[52] To date, no study has assessed in preclinical models or in a clinical setting the influence of surface topography, substrate rigidity, mechanical stimulation, or macromolecular crowding preconditioning in permanently differentiated or stem cell–only implantation. What has hampered preclinical/clinical translation and commercialization of these game-changing technologies?

Financial issues may be the first reason. There are only a few companies that manufacture bioreactor systems with the capacity to apply loads, and the systems available are not only far too expensive, but they also have limited capacity for cell expansion. Reproducibility issues may be the second reason. Although electrospinning is widely available in the laboratory setting, only a handful of companies have industrialized the process, and it is still challenging to control precisely the dimensionality of electrospun mats. Scalability issues may be the third reason. Although imprinting has solved the problem of reproducible scaffold dimensionality, we are still far away from producing economically the likely trillions of imprinted cell culture substrates required per year to expand cells for education, research, development, and clinical purposes.

Lack of sufficient evidence may be the fourth reason. Although macromolecular crowding has been available since the 1980s, only a handful of studies have assessed its potential in cell culture context. Standardization may be the fifth reason. Rarely will one find published papers reporting that authors extracted the cells in the same fashion, used the same media, applied the same preconditioning conditions, and conducted the same analysis. Regulatory issues may be the sixth reason. Most of the scaffold-based surface topography/substrate rigidity experiments are performed using non-FDA approved polymers.

Undeniably, the cell culture market is growing exponentially; it is expected to worth $18.63 billion by 2020[53] and $37 billion by 2022.[54] Unless a disruptive innovation comes along, it is likely that functional reparative therapies will involve the delivery of a relevant cell population that has been expanded in vitro. It is therefore imperative to direct

Dr. Zeugolis or an immediate family member has received research or institutional support from Viscus Biologics, Stem Cell Technologies, and NeoSurgical; has received nonincome support (such as equipment or services), commercially derived honoraria, or other non-research–related funding (such as paid travel) from Medtronic; and serves as a board member, owner, officer, or committee member of the Tissue Engineering International & Regenerative Medicine Society (TERMIS) and MBI. Neither of the following authors nor any immediate family member has received anything of value from or has stock or stock options held in a commercial company or institution related directly or indirectly to the subject of this article: Ms. Guillaumin and Mr. Sallent.

This work is supported by the: European Commission, Horizon 2020, Marie Skłodowska-Curie Actions, Innovative Training Networks (ITN) Programme Tendon Therapy Train, under the grant agreement number 676338; Health Research Board, Health Research Awards Programme, under the grant agreement number HRA_POR/2011/84; and Science Foundation Ireland, Career Development Award Programme, under the grant agreement number 15/CDA/3629. This publication has also been supported from Science Foundation Ireland and the European Regional Development Fund, under the grant agreement number 13/RC/2073.

our efforts toward the creation of physiologically/clinically relevant, industrially scalable, and regulatory compliant in vitro microenvironments in order to develop in the years to come remedial patient bedside cell-based therapies.

## References

References printed in **bold type** are those published within the past 5 years.


1. **Yelin E, Weinstein S, King T: The burden of musculoskeletal diseases in the United States. *Semin Arthritis Rheum* 2016;46(3):259-260.**
2. **Bugbee WD, Pallante-Kichura AL, Görtz S, Amiel D, Sah R: Osteochondral allograft transplantation in cartilage repair: Graft storage paradigm, translational models, and clinical applications. *J Orthop Res* 2016;34(1):31-38.**
3. **Duchman KR, Lynch TS, Spindler KP: Graft selection in anterior cruciate ligament surgery: Who gets what and why? *Clin Sports Med* 2017;36(1):25-33.**
4. **Wheelton A, Mace J, Khan WS, Anand S: Biomaterials and fabrication to optimise scaffold properties for musculoskeletal tissue engineering. *Curr Stem Cell Res Ther* 2016;11(7):578-584.**
5. **Qu D, Mosher CZ, Boushell MK, Lu HH: Engineering complex orthopaedic tissues via strategic biomimicry. *Ann Biomed Eng* 2015;43(3):697-717.**
6. Nakamura S, Katsuki M: Tendon grafting for multiple extensor tendon ruptures of fingers in rheumatoid hands. *J Hand Surg Br* 2002;27(4):326-328.
7. Reikerås O, Shegarfi H, Naper C, Reinholt FP, Rolstad B: Impact of MHC mismatch and freezing on bone graft incorporation: An experimental study in rats. *J Orthop Res* 2008;26(7):925-931.
8. Navarro-Alvarez N, Yang YG: CD47: A new player in phagocytosis and xenograft rejection. *Cell Mol Immunol* 2011;8(4):285-288.
9. Joshi N, Reverte-Vinaixa M, Díaz-Ferreiro EW, Domínguez-Oronoz R: Synthetic resorbable scaffolds for the treatment of isolated patellofemoral cartilage defects in young patients: Magnetic resonance imaging and clinical evaluation. *Am J Sports Med* 2012;40(6):1289-1295.
10. **Christensen BB, Foldager CB, Jensen J, Jensen NC, Lind M: Poor osteochondral repair by a biomimetic collagen scaffold: 1- to 3-year clinical and radiological follow-up. *Knee Surg Sports Traumatol Arthrosc* 2016;24(7):2380-2387.**
11. **Ollivere BJ, Bosman HA, Bearcroft PW, Robinson AH: Foreign body granulomatous reaction associated with polyethelene 'Fiberwire(®)' suture material used in Achilles tendon repair. *Foot Ankle Surg* 2014;20(2):e27-e29.**
12. **Piuzzi NS, Chahla J, Jiandong H, et al: Analysis of cell therapies used in clinical trials for the treatment of osteonecrosis of the femoral head: A systematic review of the literature. *J Arthroplasty* 2017;S0883-5403(17)30195-X. [Epub ahead of print].**
13. **Bara JJ, Richards RG, Alini M, Stoddart MJ: Concise review: Bone marrow-derived mesenchymal stem cells change phenotype following in vitro culture. Implications for basic research and the clinic. *Stem Cells* 2014;32(7):1713-1723.**
14. **Li P, Zhang R, Wang L, et al: Long-term load duration induces N-cadherin down-regulation and loss of cell phenotype of nucleus pulposus cells in a disc bioreactor culture. *Biosci Rep* 2017;37(2):BSR20160582.**
15. **Jansen KA, Donato DM, Balcioglu HE, Schmidt T, Danen EH, Koenderink GH: A guide to mechanobiology: Where biology and physics meet. *Biochim Biophys Acta* 2015;1853(11 Pt B 11B):3043-3052.**
16. **Ireland RG, Simmons CA: Human pluripotent stem cell mechanobiology: Manipulating the biophysical microenvironment for regenerative medicine and tissue engineering applications. *Stem Cells* 2015;33(11):3187-3196.**
17. **Spanoudes K, Gaspar D, Pandit A, Zeugolis DI: The biophysical, biochemical, and biological toolbox for tenogenic phenotype maintenance in vitro. *Trends Biotechnol* 2014;32(9):474-482.**
18. **Di Luca A, Longoni A, Criscenti G, Mota C, van Blitterswijk C, Moroni L: Toward mimicking the bone structure: Design of novel hierarchical scaffolds with a tailored radial porosity gradient. *Biofabrication* 2016;8(4):045007.**
19. de Bont LG, Boering G, Havinga P, Liem RS: Spatial arrangement of collagen fibrils in the articular cartilage of the mandibular condyle: A light microscopic and scanning electron microscopic study. *J Oral Maxillofac Surg* 1984;42(5):306-313.
20. **Biggs M, Pandit A, Zeugolis DI: 2D imprinted substrates and 3D electrospun scaffolds revolutionize biomedicine. *Nanomedicine (Lond)* 2016;11(9):989-992.**
21. **Ryan CN, Fuller KP, Larrañaga A, et al: An academic, clinical and industrial update on electrospun, additive manufactured and imprinted medical devices. *Expert Rev Med Devices* 2015;12(5):601-612.**
22. Zhu J, Li J, Wang B, et al: The regulation of phenotype of cultured tenocytes by microgrooved surface structure. *Biomaterials* 2010;31(27):6952-6958.
23. **English A, Azeem A, Spanoudes K, et al: Substrate topography: A valuable in vitro tool, but a clinical red herring for in vivo tenogenesis. *Acta Biomater* 2015;27:3-12.**
24. Noriega SE, Hasanova GI, Schneider MJ, Larsen GF, Subramanian A: Effect of fiber diameter on the spreading, proliferation and differentiation of chondrocytes on electrospun chitosan matrices. *Cells Tissues Organs* 2012;195(3):207-221.
25. **Azeem A, English A, Kumar P, et al: The influence of anisotropic nano- to micro-topography on in vitro and in vivo osteogenesis. *Nanomedicine (Lond)* 2015;10(5):693-711.**
26. **Teh TK, Toh SL, Goh JC: Aligned fibrous scaffolds for enhanced mechanoresponse and tenogenesis of mesenchymal stem cells. *Tissue Eng Part A* 2013;19(11-12):1360-1372.**
27. **Mahmoudi M, Bonakdar S, Shokrgozar MA, et al: Cell-imprinted substrates direct the fate of stem cells. *ACS Nano* 2013;7(10):8379-8384.**
28. Peng H, Yin Z, Liu H, et al: Electrospun biomimetic scaffold of hydroxyapatite/chitosan supports enhanced osteogenic differentiation of mMSCs. *Nanotechnology* 2012;23(48):485102.
29. **Lange JR, Fabry B: Cell and tissue mechanics in cell migration. *Exp Cell Res* 2013;319(16):2418-2423.**
30. **Plotnikov SV, Waterman CM: Guiding cell migration by tugging. *Curr Opin Cell Biol* 2013;25(5):619-626.**
31. **Grier WK, Iyoha EM, Harley BA: The influence of pore size and stiffness on tenocyte bioactivity and transcriptomic stability in collagen-GAG scaffolds. *J Mech Behav Biomed Mater* 2017;65:295-305.**
32. Schuh E, Kramer J, Rohwedel J, et al: Effect of matrix elasticity on the maintenance of the chondrogenic phenotype. *Tissue Eng Part A* 2010;16(4):1281-1290.
33. **Witkowska-Zimny M, Walenko K, Wrobel E, Mrowka P, Mikulska A, Przybylski J: Effect of substrate stiffness on the osteogenic differentiation of bone marrow stem cells and bone-derived cells. *Cell Biol Int* 2013;37(6):608-616.**
34. **Rehmann MS, Luna JI, Maverakis E, Kloxin AM: Tuning microenvironment modulus and biochemical composition promotes human mesenchymal stem cell tenogenic differentiation. *J Biomed Mater Res A* 2016;104(5):1162-1174.**
35. **Kwon HJ, Yasuda K: Chondrogenesis on sulfonate-coated hydrogels is regulated by their mechanical properties. *J Mech Behav Biomed Mater* 2013;17:337-346.**

36. Guvendiren M, Burdick JA: Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat Commun* 2012;3:792.

37. **Shwartz Y, Blitz E, Zelzer E: One load to rule them all: Mechanical control of the musculoskeletal system in development and aging. *Differentiation* 2013;86(3): 104-111.**

38. **Neidlinger-Wilke C, Galbusera F, Pratsinis H, et al: Mechanical loading of the intervertebral disc: From the macroscopic to the cellular level. *Eur Spine J* 2014;23(3 suppl 3):S333-S343.**

39. **Gordon R, Bloxham S: A systematic review of the effects of exercise and physical activity on non-specific chronic low back pain. *Healthcare (Basel)* 2016;4(2):E22.**

40. **Daly RM: Exercise and nutritional approaches to prevent frail bones, falls and fractures: An update. *Climacteric* 2017;20 (2):119-124.**

41. **Huisman E, Lu A, McCormack RG, Scott A: Enhanced collagen type I synthesis by human tenocytes subjected to periodic in vitro mechanical stimulation. *BMC Musculoskelet Disord* 2014;15:386.**

42. **DiFederico E, Shelton JC, Bader DL: Complex mechanical conditioning of cell-seeded agarose constructs can influence chondrocyte biosynthetic activity. *Biotechnol Bioeng* 2017. Forthcoming.**

43. Keogh MB, Partap S, Daly JS, O'Brien FJ: Three hours of perfusion culture prior to 28 days of static culture, enhances osteogenesis by human cells in a collagen GAG scaffold. *Biotechnol Bioeng* 2011;108(5): 1203-1210.

44. **Youngstrom DW, LaDow JE, Barrett JG: Tenogenesis of bone marrow-, adipose-, and tendon-derived stem cells in a dynamic bioreactor. *Connect Tissue Res* 2016;57(6): 454-465.**

45. **Cochis A, Grad S, Stoddart MJ, et al: Bioreactor mechanically guided 3D mesenchymal stem cell chondrogenesis using a biocompatible novel thermo-reversible methylcellulose-based hydrogel. *Sci Rep* 2017;7:45018.**

46. **Chen G, Xu R, Zhang C, Lv Y: Responses of MSCs to 3D scaffold matrix mechanical properties under oscillatory perfusion culture. *ACS Appl Mater Interfaces* 2017;9 (2):1207-1218.**

47. **Ravichandran A, Lim J, Chong MS, et al: In vitro cyclic compressive loads potentiate early osteogenic events in engineered bone tissue. *J Biomed Mater Res B Appl Biomater* 2016. [Epub ahead of print].**

48. **Gnutt D, Ebbinghaus S: The macromolecular crowding effect: From in vitro into the cell. *Biol Chem* 2016;397(1):37-44.**

49. **Mourão MA, Hakim JB, Schnell S: Connecting the dots: The effects of macromolecular crowding on cell physiology. *Biophys J* 2014;107(12): 2761-2766.**

50. **Satyam A, Kumar P, Fan X, et al: Macromolecular crowding meets tissue engineering by self-assembly: A paradigm shift in regenerative medicine. *Adv Mater* 2014;26(19):3024-3034.**

51. **Cigognini D, Gaspar D, Kumar P, et al: Macromolecular crowding meets oxygen tension in human mesenchymal stem cell culture: A step closer to physiologically relevant in vitro organogenesis. *Sci Rep* 2016;6:30746.**

52. Nirmalanandhan VS, Juncosa-Melvin N, Shearn JT, et al: Combined effects of scaffold stiffening and mechanical preconditioning cycles on construct biomechanics, gene expression, and tendon repair biomechanics. *Tissue Eng Part A* 2009;15(8):2103-2111.

53. Markets and Markets. *Cell Culture Market by Equipment (Bioreactor, Culture Vessels (Multiwell Plates, Petri Dish)), Consumables (FBS, ABS, Media, Reagents), Application (Therapeutics, Stem Cell), End Users (Pharmaceutical and Biotechnology, Research) - Forecast to 2020*. February 2016. http://www.marketsandmarkets.com/Market-Reports/cell-culture-market-media-sera-reagents-559.html Accessed May 9, 2017.

54. Grand View Research. *Cell Culture Market Analysis by Consumables (Media, Sera, Reagents), By Products (Culture Systems, Incubators, Pipetting Instruments, Centrifuges, Biosafety Equipment, Cryostorage Equipment), By Application (Biopharmaceuticals, Cancer Research, Drug Development, Gene Therapy, Tissue Culture & Engineering, Toxicity Testing, Vaccine Production) And Segment Forecasts To 2022*. February 2016. Report ID: 978-1-68038-536-6 http://www.grandviewresearch.com/industry-analysis/cell-culture-market Accessed May 9, 2017.